# EFFECT OF ULTRASOUND ON ENZYMATIC EXTRACTION OF ZINGIBER OFFICINALE

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## ABSTRACT

The production of essential oils from natural sources is highly profitable nowadays. The herbaceous perennial of ginger has been used as spices and is common condiment in variety of foods and beverages. Ginger is scientifically known as Zingiber officinale. This study is focused to extract the essential oil of ginger by using the technique of enzymatic extraction assisted with ultrasound in order to maximize the percentage of oil yield. Enzymatic extraction since past few decades had been acclaimed as the alternative process to produce the essential oil of herbs and oilseeds which more economically and environmental friendly than usual conventional extraction. This research has been carried out by using enzyme pectinase with different concentration from 1%, 1.5%, 2% and 3% for enzymatic extraction. The ultrasound procedure was performed by 10% and 20% duty cycle. As result indicates that by using enzymatic extraction method assisted with ultrasound, the high and good quality production of ginger oil was obtained. Pectinase concentration of 2% (v/w) yielded the highest percentage of oil recovered which is 66% and with 10% duty cycle of ultrasound, the yield was increased to 73.7% compared to solvent extraction yielded about 39% oil. As a conclusion, apart from being effective and as an alternative to conventional method, enzymatic extraction assisted with ultrasound also discovers the potential reduction of solvent consumption.

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## ABSTRAK

Pengeluaran pati minyak daripada sumber semula jadi mempunyai pelbagai keuntungan dan kegunaan pada masa kini. Herba halia telah digunakan sebagai rempah dan perasa dalam pelbagai makanan dan minuman. Nama saintifik bagi halia adalah *Zingiber officinale*. Kajian ini adalah mengenai pengekstrakan pati minyak halia dengan menggunakan teknik pengekstrakan dengan menggunakan enzim dan dibantu oleh ultrabunyi untuk peningkatan penghasilan minyak. Melalui teknik pengekstrakan enzim, sejak beberapa dekad yang lalu telah diiktiraf sebagai proses alternatif untuk menghasilkan pati minyak herba yang lebih ekonomi dan mesra alam daripada pengekstrakan konvensional. Kajian ini telah dijalankan melalui penggunaan enzim *pectinase* dengan kepekatan yang berbeza iaitu 1%, 1.5%, 2% dan 3% untuk pengekstrakan enzim. Prosedur ultrabunyi telah dilakukan dengan menggunakan 10% dan 20% kitaran. Kaedah pengekstrakan menggunakan enzim dibantu dengan ultrabunyi, telah menghasilkan pengeluaran pati minyak halia yang berkualiti tinggi dan baik. Kepekatan enzim *pectinase* sebanyak 2% telah menghasilkan peratusan tertinggi minyak iaitu sebanyak 66% dan dengan kitaran 10% ultrabunyi, penghasilan minyak telah meningkat kepada 73.7% berbanding dengan pengekstrakan konvensional di mana penghasilan minyak adalah sebanyak 39%. Sebagai kesimpulan, selain daripada alternatif dan berkesan, kaedah pengekstrakan dengan menggunakan enzim dan dibantu oleh ultrabunyi juga dapat mengurangkan kadar penggunaan pelarut dalam pengesktrakan konvensional.

## TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS/ABBREVIATIONS

| | |
|---|---|
| NSAID | Non-Steroidal Anti-Inflammatory Drug |
| UAE | Ultrasound-Assisted Extraction |
| A | Area |
| P | Power |
| °C | Degree Celcius |
| G | Gram |
| mL | Millileter |
| h | Hour |
| kHz | KiloHertz |
| s | Second |
| W | Watt |
| W $cm^{-2}$ | Watt per Centimeter Squared |
| ® | Registered Sign |
| % | Percentage |
| α | Alpha |

# CHAPTER 1

# INTRODUCTION

## 1.1 Overview of Ginger

Ginger is scientifically known as Zingiber *officinale*. The name was given by an English botanist, William Roscoe (1753-1831) in an 1807 publication. The species Zingiber officinale is said to be native from tropical climates, particularly Southeast Asia. Major world producers of ginger include Fiji, India, Jamaica, Nigeria, Sierra Leone, and China. These days broadly cultivated in India, China, Africa, Jamaica, Mexico and Hawaii. Ginger is consumed worldwide as spices and is a common condiment in variety of foods and beverages. Ginger also produce two primary extracts consist of essential oil and oleoresin. Essential oil basically gives the ginger its own unique aroma. The commercial ginger oil is normally extracted from dried rhizomes (Wohlmuth *et. al*., 2006).

## 1.2 Enzymatic Extraction

Recently, aqueous enzymatic extraction is found to be the alternative environmental friendly processes based on separation of oil and protein from oilseed, fruits, and herbs. By using specific enzyme to the extraction process, it may overcome the low extraction yields (Rosenthal *et. al*., 2001). Enzymes hydrolyze the structural polysaccharides forming the cell wall of oilseeds. Enzymes are used in extraction processes in order to assist the release of oil bodies. This kind of extraction offers many benefits, for instance, lower the investment cost and energy requirements rather than the conventional extraction (Rosenthal, et. al., 1996).

## 1.3 Ultrasound

Ultrasound has been proved to give the great impact in extraction processes. For example, it can improve the extraction yield, reduce the use of solvent, and thus to minimize the power consumption and reducing the extraction duration. The efficiency of extraction increase by ultrasound is because of the propagation of ultrasound pressure waves, and results in cavitations phenomena (Vilkhu et. al., 2008). According to Sulaiman (2011), ultrasound affects the enzyme-catalyzed reactions including formation and dissociation of the enzyme-substrate complex. Moreover, ultrasound influenced substrate and product inhibition characteristics of an enzyme.

## 1.4 Problem Statement

Enzymatic extraction since past few decades had been acclaimed by researcher as the alternative process to produce the essential oil and oleoresin of herbs and oilseeds which more economically and environmental friendly than usual conventional extraction. In addition, enzymatic extraction is to replace the solvent consumption in herbs extraction. The use of ultrasound is to gain enhancement and speed up the process of fine herbal extract, in this case is ginger. This research is going to be conduct to define the potential of ultrasound in order to enhance the performance of enzymatic extraction of ginger.

## 1.5 Objective of Research

The main objective of this research based on:

i) To obtain the best enzyme (pectinase) concentration used for enzymatic extraction of Zingiber *officinale.*

ii) To study the effect of ultrasound with various concentration of enzyme (pectinase) and various sonication regimen for extraction.

### 1.6 Research Question

i) What is the best characterization of enzyme used in enzymatic extraction of ginger oil?

ii) What is the best sonication regimen of ultrasound to produce good yield of ginger oil by enzymatic extraction?

### 1.7 Scope of Research

To achieve the objective of the study, the scope of research work is consists of as the following:

i) To determine the best enzyme concentration used in enzymatic extraction of Zingiber *officinale*.

ii) To define the best possible sonication regimen of ultrasound in order to produce good yield of Zingiber *officinale*.

### 1.8 Significant and Contribution of Research

Ginger oil has many benefits to human and also environment. The aqueous enzymatic extraction is an alternative to produce ginger oil which is more environmental friendly. Furthermore, this kind of extraction will also eliminate the solvent consumption in usual conventional extraction. Hence, potential savings in the operational cost and reduce the energy consumption. This process also has been proved in yielding a good quality of oil. By using ultrasound-assisted, the duration of extraction also can be reduced and also will generate more good products.

# CHAPTER 2

# LITERATURE REVIEW

This chapter consists of three subtopics that discuss about previous study related to the extraction of Zingiber officinale. Three subtopics consists of background information of Zingiber officinale, a range of extraction method used as well as ultrasound-assisted extraction that will help in providing information for this current research.

## 2.1 Background Information of *Zingiber officinale*

Ginger, the rhizome of Zingiber officinale, from the Zingiberaceae family is one of the extensively used spices and is a common condiment for various foods and beverages. The Zingiberaceae is a tropical family consists of several species, which are known to produce essential oils, mainly in their seeds and rhizomes. The name of Zingiber officinale was given by an English botanist, William Roscoe (1753-1831) in an 1807 publication. The name of Z. officinale comes from the Sanskrit word which by means is “horn-shaped” and it is said to be native from tropical climates, particularly Southeast Asia.

Nowadays, it is extensively been cultivated in most tropical countries for example in Australia, China, West Africa, Brazil, Japan and the West Indies (Ukeh *et. al.*, 2009). Ginger is an herbaceous perennial with upright stems and narrow medium green leaves arrayed in two ranks on each stem. Skinner (2003) found that the plant may grow to about 1.2 m tall with leaves about 1.9 cm wide and 17.8 cm long. The rhizomes are aromatic, thick lobed, branched and scaly structures with a spicy lemon-like scent. It is well known that ginger rhizomes contain both aromatic and pungent components (Singh, *et. al.*, 2008).

**Figure 1.0** Ginger Plant

### 2.1.1 Active Constituents of Ginger

The unique flavor properties of ginger come from the combination of pungency and aroma. The pungency is provided by non-volatile phenolic compounds, while the essential oil gives the ginger its own unique aroma. Ginger produce two primary extracts which are oleoresin and essential oil. Solvent extraction usually by using acetone or ethanol will yield the oleoresin which is containing both essential oil and the phenolic compounds, mainly for the pungency of ginger. The major pungent compounds in ginger, from studies of the lipophilic rhizome extracts, have generated potentially active gingerols (Govindarajan, 1982).

Ginger oleoresin is used extensively as a flavoring agent in the food and beverage industries. Commercial ginger oil is normally extracted from dried rhizomes. Typical ginger oil is characterized by the high content of by a high content of sesquiterpene hydrocarbons, mainly zingiberene (Wohlmuth *et. al.*, 2006). The West African entirely unscraped ginger is reported to have the highest essential oil content and the most pungent flavor (Ukeh, *et. al.*, 2009).

### 2.1.2 Usage of Ginger

Ginger is consumed worldwide as spices and is a common condiment in variety of foods and beverages. Ginger also been used in cosmetic industries. Ginger oil is useful in order to overcome any symptoms of food poisoning. It has antiseptic and carminative properties that are useful in curing and relieving stomach problems. In addition, it is used to improve the symptoms of nausea in pregnancy. The study by Philips *et. al*. (1993) shows that ginger reduced nausea and vomiting.

Furthermore, ginger extract has been studied as an alternative to non-steroidal anti-inflammatory drug (NSAID) therapy for arthritic conditions. This ginger rhizome is warming, thus it stimulates digestion, respiration, blood circulation and the nervous system. Ginger may be helpful in reduction of cholesterol levels and prevention of blood clotting and reduces the incidences of heart strokes. Ginger also used as an expectorant and relieves the symptoms of colds, cough and flu but not recommended for individuals with digestive ulcers, high fevers or inflammed skin conditions. Recently, ginger has been pronounced as an antioxidant, anti-inflammatory, antithrombotic and anticancer agent, also effective in reducing the symptoms of arthritis in humans (Lantz, *et. al*., 2007).

## 2.2 Extraction

Extraction is the process to remove one or more solutes from a liquid by removing the solute into a second liquid phase, for which the solute has a higher affinity (Ibrahim, 2006). Extraction depends on the disparity in both solute solubility and density of the two phases. Extraction always involves two steps. First, the solvent is contacted with the solid to be treated so as to transfer the solute to the solvent. Second, the separation or washing of the solution from the residual solid.

Liquid always adheres to the solid which must be washed to prevent either the loss of solution or the contamination loss of the solids, if these are the desired material. The complete process includes the separate recovery of the solute and solvent and can be done by evaporation or distillation. The advantages of extraction is it can be performed at ambient temperature. Thus, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules.

### 2.2.1 Solid-Liquid Extraction

Solid-liquid extraction or leaching is the process of removing a solute from a solid phase, the solid is contacted with a liquid phase for instance by using liquid solvent. The solid and liquid phases are in contact and the solute can diffuse from the solid to the liquid phase, resulting in a separation of the components originally in the solid.

Solid-liquid extraction process can be considered in three parts involving diffusion of the solvent through the pores of the solid, the diffused solvent dissolves the solutes, and transfer of the solution from porous solid to the main bulk of the solution. Leaching is widely used in chemical industries and extraction of sugar from sugar beets, oil from oil bearing seeds, production of a concentrated solution of a valuable solid material are typical industrial examples of leaching. In leaching, washing is the process when an undesirable component is removed from a solid with water (Geankoplis, 2003).

### 2.2.2 Solvent Extraction

Based on the research found by Rosenthal *et. al.* (1996), continuous solvent extraction system has been developed after the World War I, and it has been proved that the system was excellent for processing oleaginous materials with very low oil content. In these days, the modern solvent extraction process has evolved. The solvent used is hexane. The process basically by successive countercurrent washes with hexane of the previously cracked, flaked, ground, or pressed of oleaginous material for example soybean and oilseed. Then, the extracted meal is carried by a sealed conveyor for solvent recovery in enclosed vessels by using jacket or steam. Hexane is removed from the oil in rising film evaporators and finally, by vacuum distillation. For extraction using hexane as a solvent, it is possible to achieve oil yields in excess of 95% with a solvent recovery of over 95%.

Solvent extraction resulting in low extraction yields, requires long extraction times and the final product often contains traces of organic solvents, which decrease the product quality. Solvent extraction underwent the severe heat treatment during the oil extraction. The process will affect the quality of oil. Moreover, further chemical treatments are needed after the extraction to make it useful for human consumption.

High demands for oil without any chemical treatment are discovered recently. Solvent extraction may yield low oil recovery which may be overcome by utilizing selected enzymes (Latif and Anwar, 2011). Thus, the development of an effective and selective method for oil extraction is important and the potential alternative to this conventional extraction methods is enzyme-based extraction.

### 2.2.3 Aqueous Enzymatic Extraction

Enzyme-based extraction is a potential alternative to conventional solvent-based extraction methods. Aqueous enzymatic extraction is one of the alternative eco-friendly processes based on simultaneous separation of oil and protein from oilseed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid, and aqueous phases. According to Rosenthal *et. al.* (2001), the low extraction yields of aqueous processes can be defeat by using enzymes that hydrolyze the structural polysaccharides forming the cell wall of oilseeds. The presence of certain enzymes during extraction boosts oil recovery by breaking cell walls and oil bodies.

Enzymes are ideal catalysts to assist in the extraction of natural origin. Enzymes have the ability to degrade or disrupt cell walls and membranes, thus will help in releasing and more efficient extraction of bioactives, oils and protein from plants. Because of the need for eco-friendly extraction technologies nowadays, enzyme-assisted extraction has gaining more attention in worldwide. To degrade some cell walls, improve juice extractability and increase the oil yield, enzymes such as pectinases, cellulases and hemicellulases are widely used. Components such as phenolic compounds will released by the disruption of the cell wall, therefore improving product quality (Puri, *et. al*., 2012).

Pectic enzymes have been used in food processing for the extraction of oils, extraction, clarification and concentration of fruit juices, extraction of pectin and also flavours and pigments from plant materials. For oil extraction, enzymes which is most used for oil extraction are cellulase, α-amylase and pectinase. According to Rosenthal, *et. al.* (1996), enzymes pectinase and cellulase are the most effective for increasing oil yields of olives. While for avocado, the better extraction yields by using α-amylase alone and resulting 75% of the original content of oil in comparison to 65% with the combination of polygalacturonase, α-amylase and protease. By using mixture of cellulase and pectinase, an increase to 30% in the sunflower aqueous extraction yield is discovered by Dominguez, *et. al.* (1995). It is a very effective approach for oil extraction of coconut, soybean, and corn germ, yield the oil recovery in the range of 90–98% and good quality protein meal (Sharma, *et. al.*, 2002). The past studies about the enzymatic aqueous extraction for different oil-bearing materials in comparison to control are shown in Table 2.1.

**Table 2.1** Enzymatic aqueous extraction for different oil-bearing materials in comparison to control (without enzyme)

| Material | Enzyme | Concentration | Oil Yield (%) | Reference |
|---|---|---|---|---|
| Rapeseed | Control<br>Pectinase (Pectinase Ultra-SP) | -<br>2% | 53.9<br>71.4 | Deng, *et. al*. (1992) |
| Avocado | Control<br>α-Amylase + cellulase | -<br>1% | 2.0<br>67.0 | Buenrostro and Lopez-Munguia (1986) |
| Sunflower | Control<br>Cellulase (CGA) | -<br>3% | 30.0<br>44.0 | Lanzani, *et. al.* (1975) |
| Soybean | Control<br>Protease (Sigma) | -<br>0.2% | 62.0<br>86.0 | Yoon, *et. al.* (1991) |
| Peanut | Control<br>Protease (pepsin-Merck) | -<br>3% | 72.0<br>78.0 | Lanzani, *et. al.* (1975) |

Aqueous enzymatic oil extraction offers many benefits compared to conventional extraction. The process will eliminate the solvent consumption which reportedly may also lower the investment cost and energy requirements. Furthermore, it allows simultaneous recovery of oil and protein from most oilseeds and the process yields oil of good quality complying with Codex specifications (Rosenthal, *et. al.*, 1996). Recent studies by Puri, *et. al*. (2012) shown that, the enzyme-assisted extraction method will achieve high extraction yield for compounds for instance oils, natural pigments, flavour and medicinal compounds. Furthermore, this kind of extraction will resulting in faster extraction and higher recovery when compared to non-enzymatic methods.

## 2.3 Ultrasound-Assisted Extraction

Ultrasound has been widely used as an assisting extraction from plant material since few past decades. Ultrasound technology has been proved wherein offer many advantages in extraction, for instance, increasing the extraction yield, reducing solvent usage, economizing power consumption and reduce the duration of extraction. The efficiency of extraction increase by ultrasound is because of the propagation of ultrasound pressure waves, and results in cavitation phenomena (Vilkhu *et. al.*, 2008). The improvement of extraction process can be made with the disruption of cell walls and the release of cellular materials (Patist & Bates, 2008). Moreover, ultrasound-assisted extraction (UAE) may performed at a lower temperature, thus, avoiding thermal damage to the extracts and minimize the loss of